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The Agar Disc Method for Studying the Contamination from Metal Surfaces

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DAIRY INDUSTRY SECTION

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The Agar Disc Method for Studying the Contamination from Metal Surfaces[1]

By H. C. Olson and B. W. Hammer

A procedure, designated the agar disc method, has been developed at the Iowa Agricultural Experiment Station[2] for the study of the contamination from churns. It consists of allowing a small amount of a special agar medium to solidify in contact with the surface to be studied, the transferring of the disc thus formed to a sterile petri dish and, finally, the counting of the colonies that develop on incubation. The usefulness of the method for the examination of churns, especially when the churns are at some distance from the laboratory[3], suggests its application to the study of the contamination from metal utensils and equipment. Trials on milk cans, vats, coolers, bottlers, freezers, sanitary piping, etc., indicate that the agar disc method is readily applicable to metal surfaces.

GENERAL STEPS IN THE AGAR DISC METHOD WHEN USED ON METAL SURFACES

a. After melting and cooling to approximately 43° C., the special medium (about 10 ml.) is poured on a small area of the surface to be examined and allowed to solidify. The medium that has been used is beef infusion agar made with 2.5 percent of air dried agar so that the disc will be strong enough to permit handling.[2] Other media can be employed and perhaps are advisable for some purposes.

b. The agar disc is picked up with a sterile spatula and tipped into a sterile petri dish so that the portion of the disc that was in contact with the surface being examined is toward the top of the dish.

c. The disc is incubated at 21° C. for 4 days, although if the disc is so heavily seeded that there is danger of the colonies fusing, the incubtion period is shortened. The incubation conditions can be varied for special purposes.

d. The colonies developing on a measured area (usually 20 to 30 sq. cm.) are counted and the results expressed as the number per square centimeter. With very heavily seeded discs, either approximate counts or estimates are accepted.

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[1] Project No. 126 of the Iowa Agricultural Experiment Station.

[2] B. W. Hammer and H. C. Olson. Bacteriology of butter III. A method for studying the contamination from churns. Ia. Agr. Exp. Sta., Res. Bul. 141. 1931.

[3] H. C. Olson and B. W. Hammer. Bacteriology of butter V. Studies on the microorganisms in churns. Ia. Agr. Exp. Sta., Res. Bul. 159. 1933.

GENERAL OBSERVATIONS ON THE USE OF THE AGAR DISC METHOD ON METAL SURFACES

The agar disc method is readily applied to metal surfaces because the discs are so easily removed. It is not necessary to moisten the area to be covered, as is the case with wood surfaces.[4] On metal surfaces that are approximately horizontal, the size and shape of a disc can be controlled in a general way by careful pouring of the medium. Utensils or small pieces of equipment can be tilted so that a nearly horizontal surface is secured. If the surface to be examined is thoroughly cooled, the medium will solidify very quickly, but if the surface is rather warm the medium may spread over a large area before solidifying and the disc be too thin to be easily removed.

When the surface to be examined is completely covered with water, the disc secured is commonly too large and too thin to withstand the necessary handling, but droplets of water do not interfere with the preparation of a disc. A disc that is approximately circular and about 7 cm. in diameter can be handled conveniently.

A spatula with a thin flexible blade that is rather large and rounded at the tip is very satisfactory for transferring discs of the usual size and shape; a small spatula is more convenient for rather inaccessible places.

In several trials a number of discs were prepared as rapidly as possible on the same area. The last disc secured in a trial still showed colonies on incubation, although the number was much smaller than with the first disc. From these results it is evident that a disc picks up only a part of the organisms so that the results are of value primarily for comparative purposes. It appeared that, in general, a larger portion of the organisms present was picked up when a disc was prepared on metal than when it was prepared on wood. In the interpretation of results it should be recognized also that undoubtedly many of the colonies represent clumps of organisms rather than only single cells.

RESULTS SECURED

The agar discs that have been prepared on metal utensils and equipment in use in dairy plants indicate that the sanitary condition of the surfaces examined varies widely. The general results secured can best be shown by a series of illustrations.

A disc prepared on a metal surface that has been washed but given no treatment to destroy the organisms left on it often shows large numbers of colonies. This is illustrated by fig. 1 which shows discs from cans that had been washed in a tank

[4] B. W. Hammer and H. C. Olson. Bacteriology of butter III. A method for studying the contamination from churns. Ia. Agr. Exp. Sta., Res. Bul. 141. 1931.

of water previously used to wash a considerable number of cans.

The influence of thorough steaming on the organisms on contaminated surfaces is evident from figs. 2 and 3. Figure 2 shows a disc from a washed but unsteamed can and also a disc from the same can after steaming, while fig. 3 shows a preparation from washed but unsteamed sanitary piping and also a preparation from the piping after it had been steamed. The much smaller number of organisms per square centimeter on the unsteamed piping than on the unsteamed can is undoubtedly due to the fact that the water used to wash the can had been employed previously to wash a considerable number of other cans, while that used to wash the piping had simply been run through a small amount of other equipment. The molds on the disc from the unsteamed piping indicate that the temperature of the wash water was not especially destructive of the organisms present. Occasionally discs prepared on recently steamed equipment show considerable numbers of organisms that belong to the genus Bacillus. Figure 4 illustrates such a disc that was secured on a cooler trough. Presumably the organisms on the disc very largely survived the heat treatment; some of them may represent air contamination, but the flora of the disc is not of the type that usually results from air contamination. A cooler is difficult to steam thoroughly and there is a greater opportunity for the survival of organisms on it than on some other pieces of equipment.

The influence of exposing metal surfaces to air contamination is evident from figs. 5, 6 and 7. Figure 5 represents a disc from a can cover that had been exposed to the air for 24 hours following thorough steaming, fig. 6 shows a disc from a freshly steamed can cover and another from the same cover after exposure to the air for 24 hours, while fig. 7 represents a disc from the hopper of a freezer that had stood for some time. The discs secured from surfaces exposed to air contamination regularly show a variety of organisms with molds especially conspicuous. A disc prepared on a freezer head that had been exposed to air contamination for only a short time following steaming is shown in fig. 8; the predominance of molds is especially conspicuous.

Figure 9 illustrates the development of organisms in moist cans. One of the discs was prepared on a freshly steamed can that was covered before the moisture could escape, while the other was prepared on the same can after it had stood covered for 24 hours. At the time the second disc was prepared there were small moisture droplets over much of the inner surface of the can. The organisms on the second disc are of comparatively few types; the tendency for the colonies to be in clumps or chains is presumably due to the incomplete mixing of the moisture on the metal with the agar. Figure 10 shows a disc on

which, because of the few types present, the organisms are presumably the result of growth on the metal, although they might have survived the treatment given; the disc was prepared on a cooler trough of unknown history. An agar preparation on the bottom of a piece of sanitary piping is shown in fig. 11, and an agar disc on an inverted can cover is illustrated in fig. 12.

ADVANTAGES OF THE METHOD

The agar disc method gives a general picture, from the standpoint of the numbers, distribution and types of the microorganisms present, of the surface to which it is applied. For many persons this picture is more impressive than counts secured on milk or water used to rinse utensils or equipment. The method is readily applicable to field work because (a) comparatively little material and equipment are required and these are easily transported and (b) comparatively little work is involved in the use of the method. It should be recognized that agar discs cannot be prepared in very inaccessible places where proper cleaning is especially difficult and cannot detect contamination at points away from the surface with which the agar comes in contact, for example, in seams and cracks.

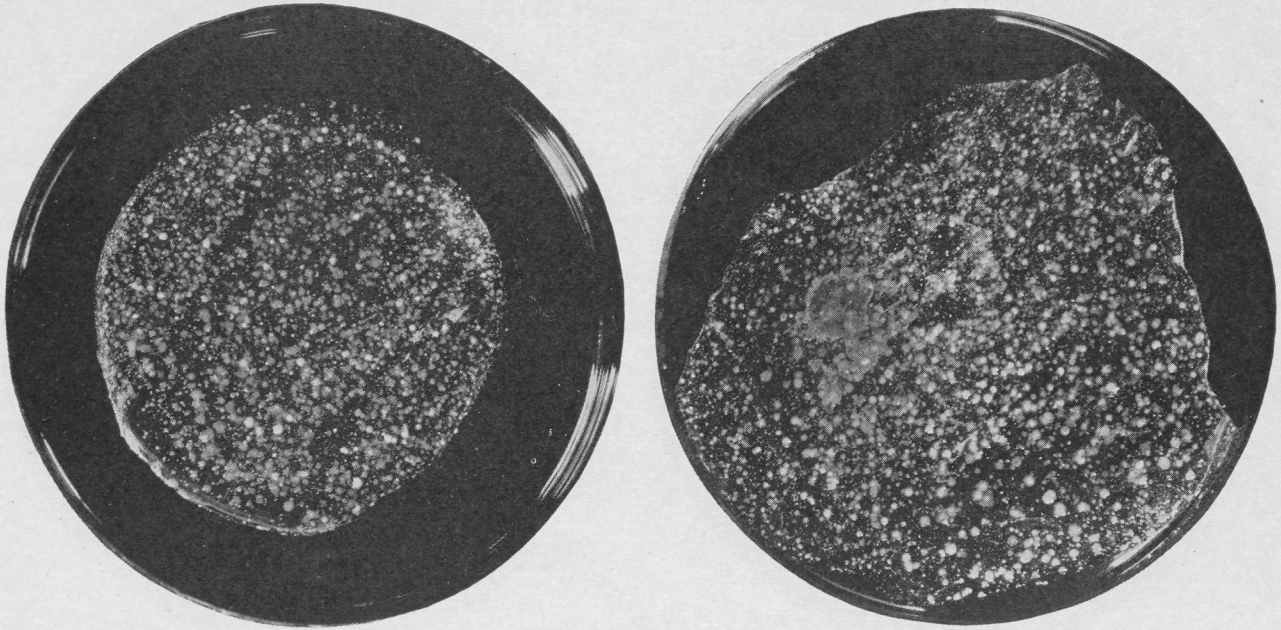

Fig. 1. Discs prepared on cans washed in water previously used to wash a considerable number of cans.

Fig. 2.

A. Disc from a washed but unsteamed can.

B. Disc from the same can after steaming.

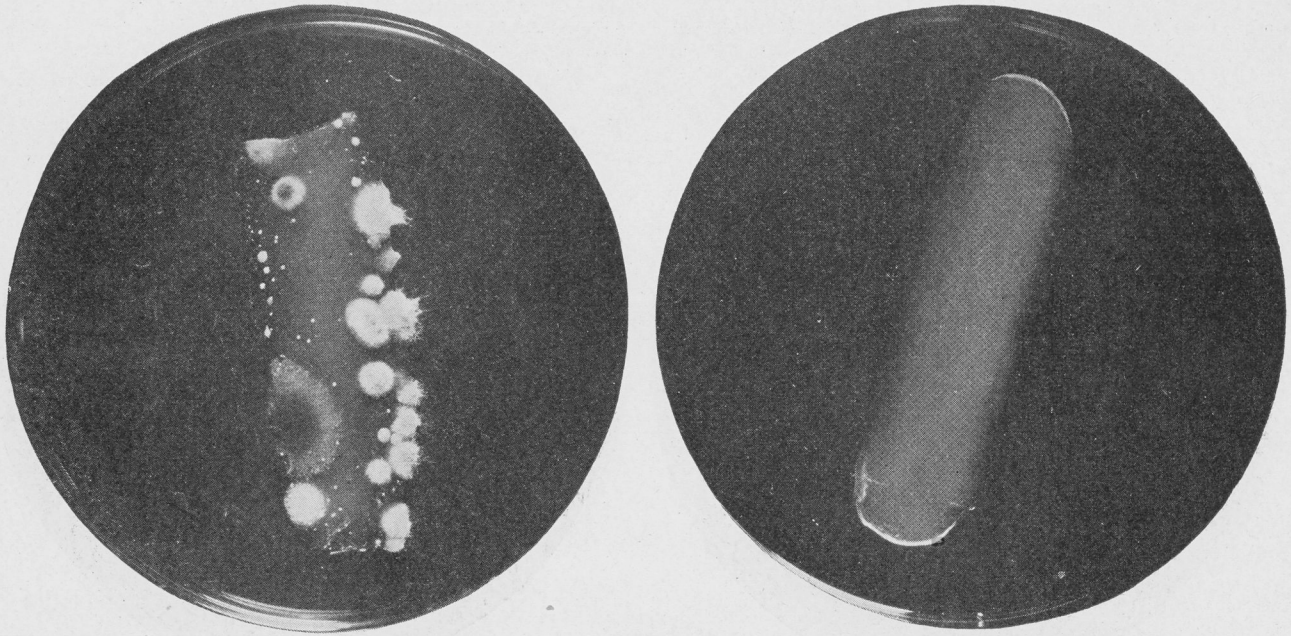

Fig. 3.

A. Preparation from washed but unsteamed sanitary piping.

B. Preparation from same piping after steaming.

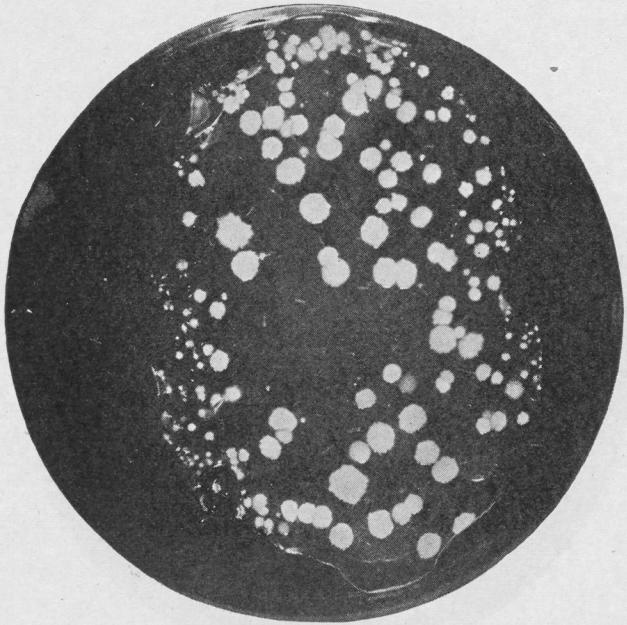

Fig. 4. Disc from a recently steamed cooler trough. The organisms belong very largely to the genus Bacillus.

Fig. 5. Disc from a can cover exposed to the air for 24 hours following thorough steaming.

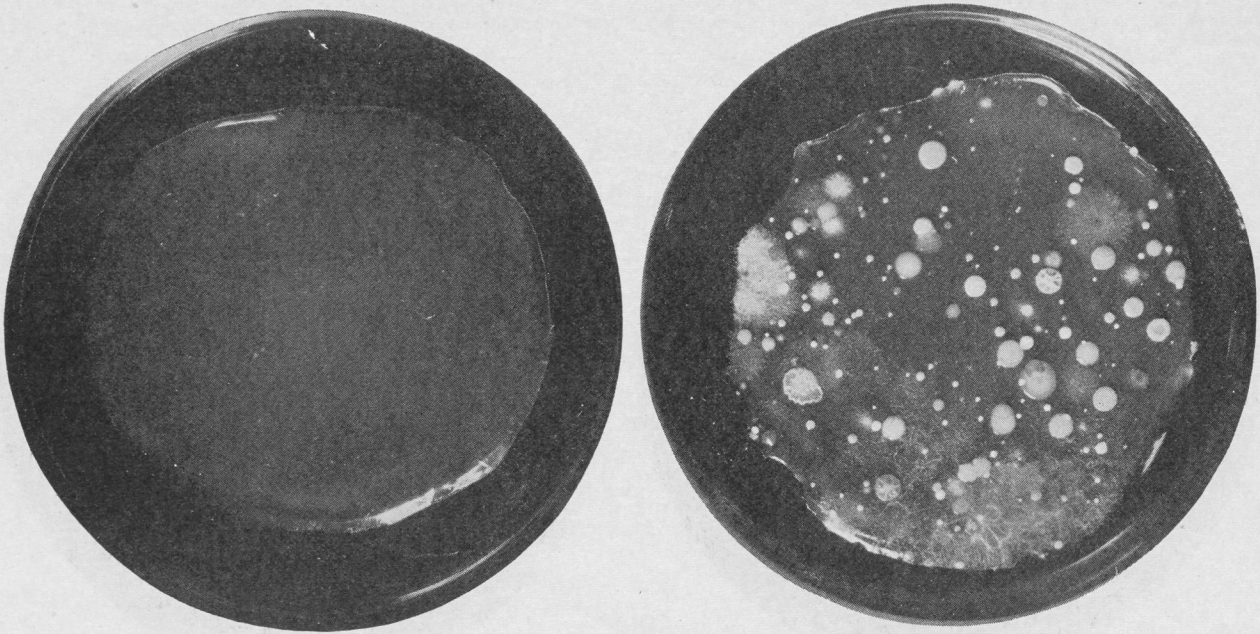

Fig. 6.

A. Disc from a freshly steamed can cover.

B. Disc from the same cover after exposure to the air for 24 hours.

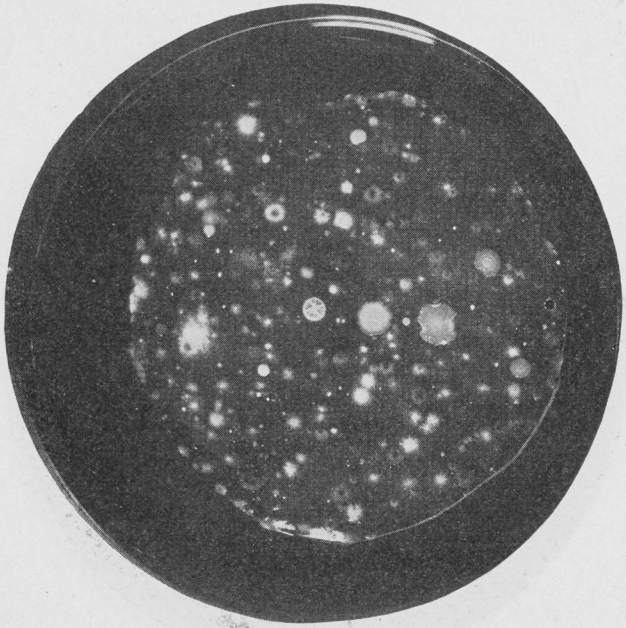

Fig. 7. Disc from the hopper of a freezer that had stood exposed for some time.

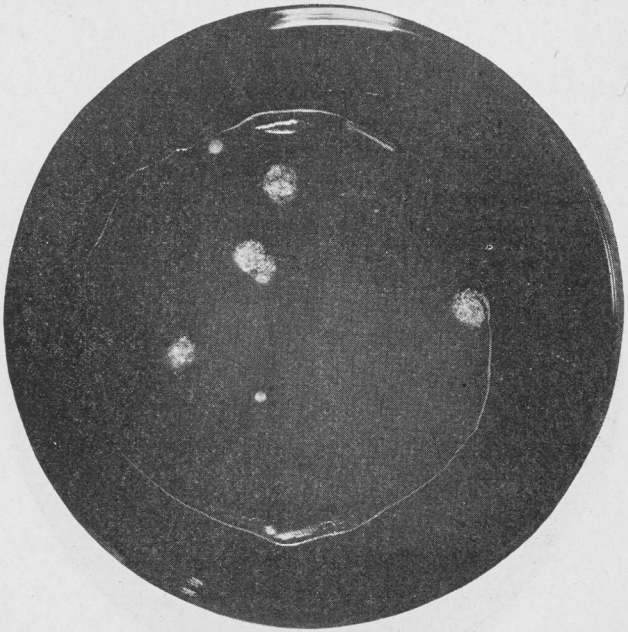

Fig. 8. Disc prepared on a freezer head that had been exposed to air contamination for only a short time following steaming.

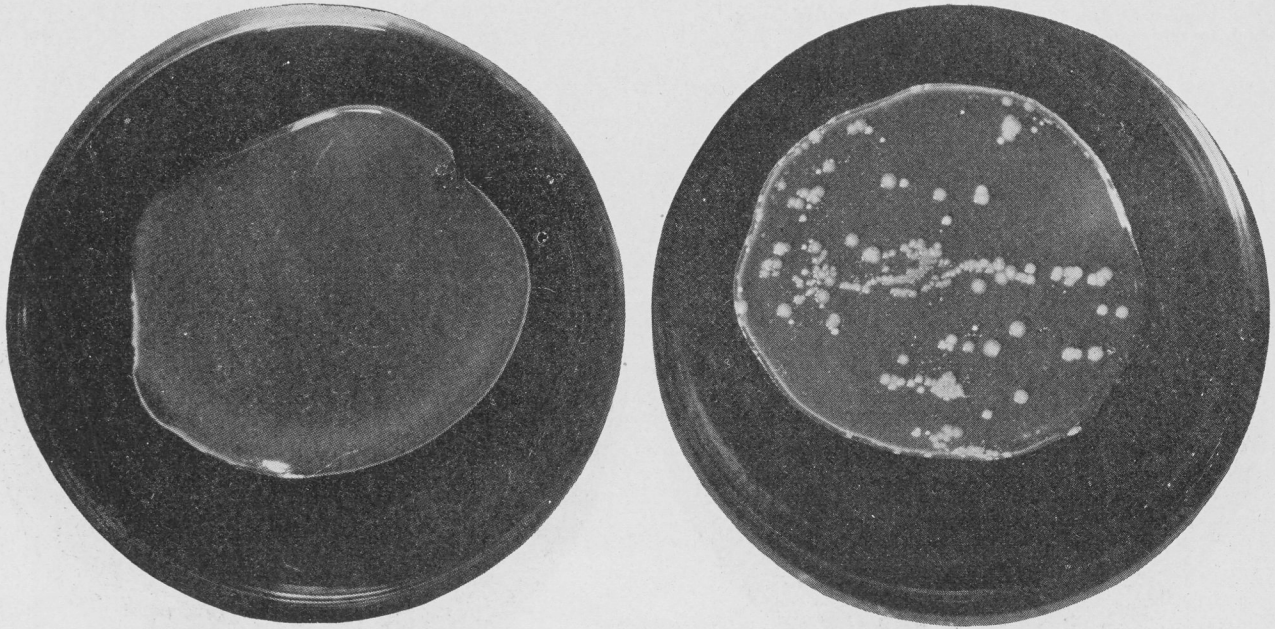

Fig. 9.

A. Disc from a freshly steamed can that was covered before the moisture could escape.

B. Disc from the same can after standing covered for 24 hours.

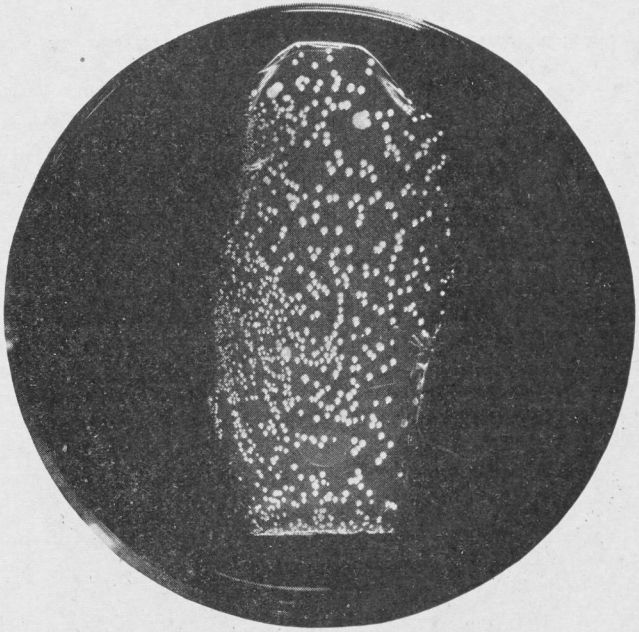

Fig. 10. Disc prepared on a cooler trough of unknown history. Because of the few types present the organ.sms presumably are the result of growth, although they might have survived the treatment given the cooler.

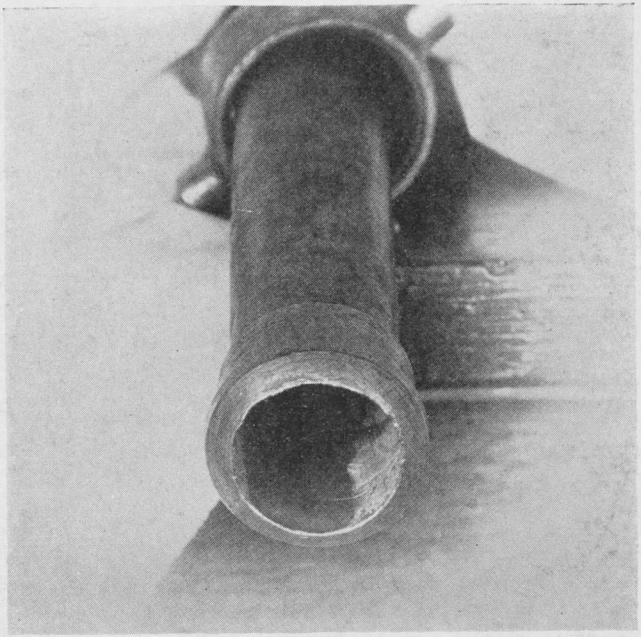

Fig. 11. Agar preparation on the bottom of a piece of sanitary piping.

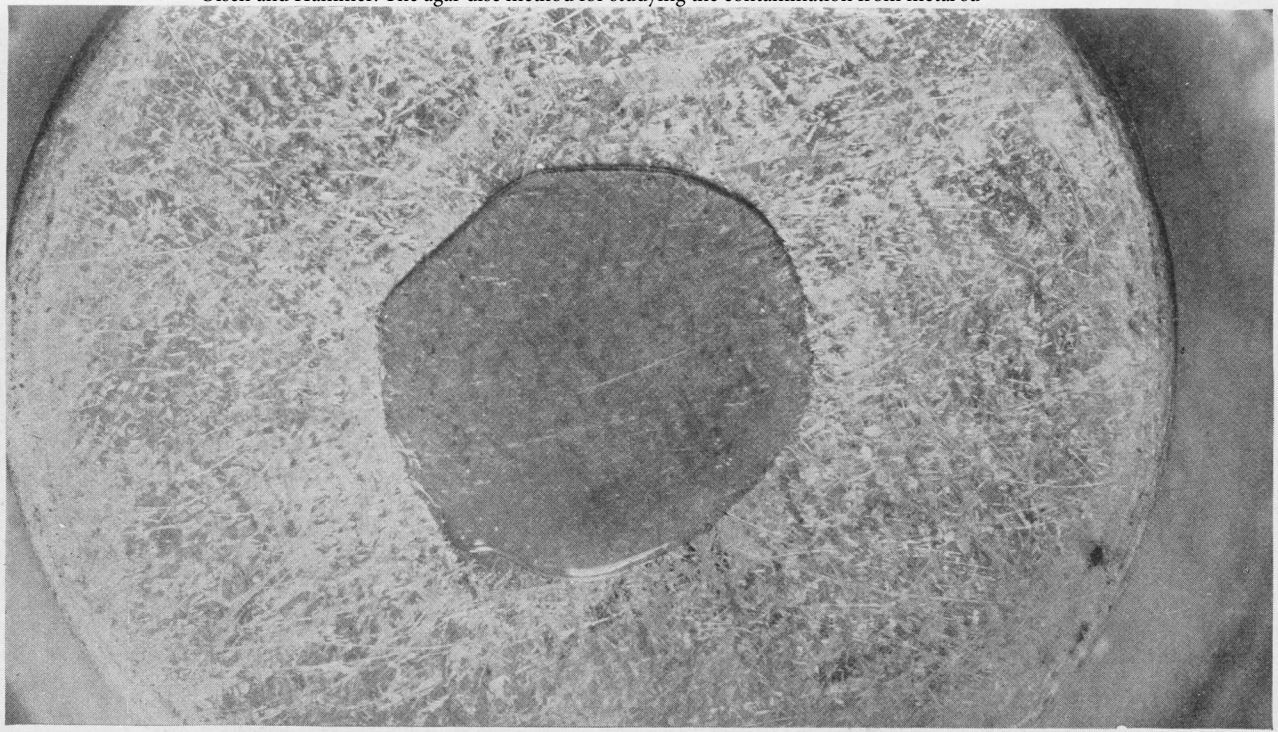

Fig. 12. Agar disc on an inverted can cover.